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Associations of Growth Hormone Gene Polymorphisms with Milk Production Traits in South Anatolian and East Anatolian Red Cattle

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Abstract: The current study was undertaken to determine the relationship between milk production traits of Eastern Anatolian Red (EAR) and South Anatolian Red (SAR) breed cows and polymorphisms of Growth Hormone gene (GH) which is a potentially effective Quantitative Trait Loci (QTL) on milk production traits. Fifty cows that were newly delivered calves from each of EAR and SAR breeds were used. Triplicate milk samples were obtained between 0-30, 50-180 and 270-300 days of lactation period. Milk samples were analyzed for milk fat, protein, dry substance, refraction indices and somatic cell count. In addition, DNA samples were obtained from blood samples of each cow and *Alu*I and *Msp*I polymorphisms in GH were determined using PCR-RFLP method. In both breeds, *Alu*I polymorphism with VV genotype cows had higher milk fat percentage compared to other genotypes. Similarly, in SAR cows, those with *Msp*I polymorphism and -/- genotype had higher milk fat percentage compared to other genotypes. The relationship between GH gene polymorphisms and other milk quality parameters could not be established. As a result, it can be concluded that GH gene polymorphisms can be of a valuable parameter to be used for selection of EAR and SAR cows for improving milk fat percentage.

Key words: Turkish native cattle breeds, growth hormone, PCR-RFLP, polymorphisims, milk production traits

INTRODUCTION

Up to date, a number of Quantitative Trait Loci (QTL), which were found effective on milk production traits were determined in cows as a result of genome screening studies . The objective of a cattle breeding program is to determine the gene sets that positively affect production traits and to improve phenotypic structures by means of transferring these gene sets to the next generations. These studies focused particularly on milk production traits (Ashwell *et al*., 2004; Schrooten *et al*., 2000).

Growth Hormone gene (GH) has been widely studied in livestock because of its effects on important biological functions including growth, body composition and development of mammary cell, lactogenesis and proliferation of mammary cells (Horvat and Medrano, 1995; Nielsen *et al*., 1995; Lagziel *et al*., 1999). GH gene of cattle is approximately 1800 bp in size and contains 5 exons and 4 introns. This gene encodes a mRNA in the size of 786 bp (Woychik *et al*., 1982). GH is located on 19th chromosome in q26-qter band region (Hediger *et al*., 1990).

Although, a number of polymorphisms were determined in GH gene of cattle up to date, 2 polymorphisms located in the intron 3 and exon 5 were found significant for their effects on milk and meat yield parameters by RFLP (Hoej *et al*., 1993; Lucy *et al*., 1991). The polymorphisms which is digested by *Msp*I restriction enzyme is located on the intron 3 (Zhang *et al*., 1993a). As a result of digestion with this enzyme, 2 alleles occur and *Msp*I (-) contains a T-insertion at +837 position and a C-G transition at +837 position (Lee *et al*., 1994a). Zhang *et al*. (1993b) reported that the polymorphism in the exon 5 could be digested by *Alu*I enzyme and 2 alleles as L and V occur. As a result of this polymorphism, leucine amino acid in the codone 127 of GH is converted to valine.

In the present study, it was aimed to investigate the relationship between polymorphisms of GH gene and milk production traits of EAR and SAR cows, 2 of the native cattle breeds of Turkey and to evaluate the use of these polymorphisms as a selection marker in these cattle breeds.

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MATERIALS AND METHODS

Animal material: In the present study, 50 dairy cows from each of EAR and SAR breeds were used. A questionnaire carried out with animal owners were used for selection of the cows. Those cows that are not relatives of each other and are exposed to similar environmental conditions and nutritional regime, are healthy and within the 0-30 days of lactation period were selected.

Milk samples and analysis: Triplicate milk samples were collected from the cows at the beginning (0-30 days), middle (150-180 days) and at the end (270-300 days) of lactation. The samples were analyzed for milk fat, solids non-fat and protein levels using a rapid milk analyzer (EKOMILK milk analyzer, EON Trading LLC, USA). Somatic cell count was determined using somatic cell counter (firefly, Charm Sciences Inc, USA) and commercial test kits. Refraction indices of milk fat were determined by a hand refractometer at the time of sample collection.

DNA isolation: Blood samples were collected in sterile 2 mL tubes containing EDTA. Genomic DNAs were isolated using a standard salt-out method (Miller *et al*., 1998).

PCR-RFLP analysis: The PCR for *Alu*I and *Msp*I polymorphisms were carried out in a final volume of 25 μL containing 1 U Taq DNA polymerase (Fermentas Life Sciences, Canada), 2-2.5 μL 10XPCR buffer (750 mM Tris HCl (pH 8.0), 200 mM $(NH_4)_2SO_4$,0.1% Tween 20), 1.5 mM $MgCl_2$, 50-100 ng genomic DNA, 100 μM dNTP (Takara, Biotechnology Co, Ltd, Japan) and 10 pmol of each primer. The Primer sequence used for the GH *Alu*I site: Primer forward: 5 GCTGCTCCTGAGGGCCCTTCG 3 and Primer reverse: 5GCGGCGGCACTTCATGACCCT 3 (Mitra *et al*., 1995). PCR conditions were 5 min at 94°C, 60 sec at 94°C, 60 sec at 60°C, 60 sec at 72°C, 32 cycles and 10 min at 72°C. The amplified samples were subjected to digestion with 10U *Alu*I at 37°C for 3 h. The resulting products were loaded to 3-5% Nusieve GTG agarose gel within 1X TBE and then run at 120 V for 40 min for separation of the DNA fragments. The bands were stained with ethidium bromide prior to visualization by UV light. The Primer sequence used for the GH *Msp*I site: Primer forward: 5′AGAATGAGGCCCAGCAGAAATC 3′ and Primer reverse: 5′GTCGTCACTGCGCATGTTTG 3′ PCR conditions were 2 min 15 sec at 94°C, 45 sec at 94°C for 5 min, 60 sec at 58°C, 60 sec at 72°C, 33 cycles and 5 min at 72°C. The amplified bands were subjected to 5 U *Msp*I at 37 °C for 16 h. The resulting products were loaded to 3-5% Nusieve GTG agarose gel within 1X TBE and then run at 120 V for 30 min for separation of the DNA fragments. The bands were stained with ethidium bromide and visualized under UV light.

Statistical analysis: Distribution of GH genotype and alleles in EAR and SAR and chi-square (χ^2) test to indicate the populations were in Hardy-Weinberg equilibrium were evaluated by using PopGene32 software (Yeh *et al*., 2000). The differences in milk protein, fat, dry substance, somatic cell count and refraction indices genotypes in both races were compared by using General Linear Model (GLM) (SPSS, version 11.5). The results are reported as mean±Standard Error (SE).

RESULTS

Detection of PCR-RFLP polymorphism: As a result of digestion of 223 bp target region on GH gene exon 5 by *Alu*I enzyme, the samples with 223 bp fragment (uncut) were accepted as VV genotype, those with 223, 171 and 52 bp fragments as VL and those with 171 and 52 bp were evaluated as LL genotypes. As a results of the 768 bp target region on intron 3 of GH gene by *Msp*I enzyme, the samples with 612, 93 and 63 bp fragments were accepted as $Msp^{(+/+)}$ genotype, those with 705, 612, 93 and 63 bp fragments were $Msp^{(+/-)}$ genotype and those with 705, 93 and 63 bp fragments were evaluated as $Msp^{(-/-)}$ genotypes.

Distribution of genotypes and alleles of GH *Alu*I and *Msp*I polymorphisms of EAR and SAR cows are provided in Table 1. In SAR cows, L allele frequency for *Alu*I polymorphism was found higher than V allele frequency. On the other hand, in EAR cows, the frequency of V alleles was higher than L alleles. As for the *Msp*I polymorphisms, Msp^+ allele frequency was appreciably higher than Msp^- allele frequency in both cattle breeds.

Milk production traits: Distribution of milk production traits by GH *Alu*I and *Msp*I polymorphism genotypes for EAR and SAR cows are given in Table 2 and 3, respectively. In both breeds, it was found that LL genotype of *Alu*I polymorphism has significantly higher fat level compared to VV genotypes ($p<0.05$). Similarly, $Msp^{(-/-)}$ genotype of *Msp*I polymorphism of SAR cows had significantly higher milk fat compared to $Msp^{(+/+)}$ genotypes ($p<0.05$). In EAR cows, however, no animal was detected as $Msp^{(-/-)}$ genotype. For other milk traits, no significant difference between the genotypes of these 2 polymorphisms was detected.

Table 1: Distribution of *GH AluI* and *MspI* polymorphisms genotypes and allele frequencies in South Anatolian and East Anatolian Red cattle

			Allel frequency (%)		Genotype		
Locus	Breed	*n*	V	L	VV	VL	LL
Alu I	SAR[1]	50	0.44	0.56	7	30	13
	EAR[2]	50	0.57	0.43	10	37	3
			+	-	+/+	+/-	-/-
Msp I	SAR	50	0.67	0.33	19	29	2
	EAR	50	0.59	0.41	9	41	0

[1]South anatolian red cattle, [2]East anatolian red cattle

Table 2: Least square means (±SE) of milk production traits in South Anatolian and East Anatolian Red cattle breeds with different GH *AluI* genotypes

	SAR[1]			EAR[2]		
Genotype	VV (n = 7)	VL (n = 30)	LL (n = 13)	VV (n = 10)	VL (n = 37)	LL (n = 3)
Fat (%)	2.78±1.96[b]	2.97±3.41[b]	4.81±3.90[a]	3.23±0.65[b]	4.45±2.26[b]	5.19±3.95[a]
Prot[3] (%)	3.84±1.84	3.09±0.64	2.94±0.50	3.10±0.39	3.35±0.40	3.26±0.57
SNF[4](%)	8.46±0.68	8.40±0.99	8.22±1.22	8.41±0.39	8.95±1.31	9.05±0.52
R[5] (%)	12.20±5.56	9.62±2.17	10.95±2.09	9.42±1.85	11.40±2.17	9.55±2.33
SCS[6] (×10[3])	128.20±50.16	130.38±75.01	142.13±100.32	105.98±67.01	113.64±54.32	100.12±98.51

[1]South Anatolian red cattle, [2]East Anatolian red cattle, [3]Protein, [4]Solids non fat, [5]Refraction indices, [6]Somatic cell score, [a,b]Means within the same line with different letters differ ($p<0.05$)

Table 3: Least square means (±SE) of milk production traits in South Anatolian and East Anatolian Red cattle breeds with different GH *MspI* genotypes

	SAR[1]			EAR[2]		
Genotype	++ (n = 9)	+- (n = 29)	-- (n = 2)	++ (n = 9)	+- (n = 41)	-- (n = 0)
Fat (%)	3.64±3.31[b]	3.96±2.30[b]	7.26±3.90[a]	4.98±2.78	5.69±1.94	-
Prot[3] (%)	3.34±1.27	3.05±0.64	3.37±0.60	3.39±0.81	3.28±0.29	-
SNF[4] (%)	8.45±1.03	8.27±0.96	8.78±1.98	9.03±2.15	8.94±0.98	-
R[5] (%)	11.02±3.64	9.96±2.70	10.20±1.98	11.02±3.17	10.98±2.19	-
SHS[6] (×10[3])	118.20±58.69	123.26±45.81	112.13±89.09	150.98±45.36	138.98±67.36	-

[1]South Anatolian red cattle, [2]East Anatolian red cattle, [3]Protein, [4]Solids non fat, [5]Refraction indices, [6]Somatis cell score, [a]Means within the same line with different letters differ ($p<0.05$)

DISCUSSION

Turkey is appreciably rich in cattle population where as quiete poor in terms of yield per animal. It is generally accepted that causes of poor yield of animals are poor environmental conditions, poor nutritional regimes as well as low capacity genotypes. However, it is clear that there is almost no published study on known potential QTLs of EAR and SAR breed cattle. SAR cattle are commonly raised in Southern Anatolia in Turkey. In addition, different varieties of this breed can be found in Syria, Israel and Egypt. Annual milk yield of SAR varies between 1000 and 1500 kg. It has been reported that annual milk yield could be as high as 5000 kg when the environmental and nutritional conditions were improved. EAR breed cattle are common in Eastern Anatolia. Annual milk yield of this breed varies between 1000 and 1200 kg. Both of these breeds are known for their ability to their adaptation to poor environmental and nutritional conditio. Milk production traits of cows are controlled by many genes as well as influenced by environmental conditions. In recent years, a number of candidate genes affecting milk production genes has been found and many studies has been undertaken investigating the relationship between these candidate genes and lactation performance of cows (Dybus *et al*., 2004).

Growth hormone is a potential Quantitative Traits Loci (QTL) affecting the milk production traits. Sabour *et al*. (1997) reported for Holstein cows that GH gene *AluI* polymorphism V allele genotypes had better milk traits, especially higher milk protein, compared to the other genotypes. In contrast Chung *et al*. (1996) reported that cows with LL genotype had higher milk protein level. Likewise, Dybus (2002a) reported that LL genotype cows had higher milk yield and milk protein. In addition, GH release in L genotype German Black-White cows was reported to be higher than V genotypes (Lucy *et al*., 1991). In the present study, LL homozygote EAR and SAR cows had significantly higher milk fat level compared to the other genotypes. Although this finding was in agreement with those reported by Dybus (2002b) regarding milk fat level, no positive relation was found between milk protein and genotypes. Hoej *et al*. (1993) and Lee *et al*. (1994a) reported that milk fat level of GH *MspI* $^{(-)}$ genotype animals was higher. Lagziel *et al*. (1999) reported that milk protein content was higher in

heterozygote animals while Dybus *et al*. (2002) reported milk fat levels was higher in *Msp*$^{(+/+)}$ homozygote animals. Unlike these findings, Yao *et al*. (1996) reported that GH *Msp*I$^{(+)}$allele positively affected milk yield, protein and fat in cows. In the current study, similar to the findings reported by these researchers, milk fat level of *Msp*$^{(-/-)}$ genotype SAR cows was significantly higher than that of the other genotypes. *Msp*I$^{(-)}$ allele frequency is usually found at low level in high yield northen European/USA and eastren European/Mediterranean cattle breeds (0.10-0.39, respectively) where as found at higher frequency in zebu cattle breeds (0.87) (Hoej *et al*., 1993; Dybus, 2002b; Lagziel *et al*., 2000). In the study, *Msp*$^{(-)}$ allel ferequancy was detected 0.33 and 0.41 in SAR and EAR cattle breeds, respectively.

CONCLUSION

As a result, we can conclude that GH gene *Alu*I polymorphism VV genotype positively affect milk fat percentage in EAR and SAR cattle. The *Msp*I polymorphism *Msp*$^{(-/-)}$ genotype also positively affect milk fat percentage in SAR. Our findings on GH gene polymorphisms can be used in as selection markers for improving the milk fat level in both Turkish native cattle breeds.

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